

Factors influencing the flux of carbon through ectomycorrhizal mycelium forming inter plant connections

R.D. FINLAY, B. SÖDERSTRÖM ⁽¹⁾ and D.J. READ

University of Sheffield, Department of Botany,
Sheffield S10 2TN, Great Britain

INTRODUCTION

In natural plant communities the root systems of a number of species may grow in close proximity to each other and the low host specificity of many mycorrhizal fungi enables their vegetative mycelia to form a network of hyphal connections which link both intra-specific and inter-specific combinations of host plants. The functional significance of such a network, in terms of its capacity to act as a direct pathway for inter-plant nutrient transfer, has been considered by a number of workers (BJORKMAN, 1960; WOODS & BROCK, 1964; HIRREL & GERDEMANN, 1979; CHIARIELLO *et al.*, 1982), however, only indirect evidence of such a pathway is available from these studies. More detailed studies by FRANCIS & READ (1984) have provided direct autoradiographic evidence of mycelial transfer of labelled assimilate between VAM plants and quantitative data from their experiments indicate that the magnitude of such transfer is strongly influenced by the shading of 'receiver' plants, which suggests that the transfer of assimilate occurs along concentration gradients influenced by source-sink relationships. REID & WOODS (1969) have demonstrated transfer of ¹⁴C labelled assimilate between adjacent seedlings of *Pinus taeda* artificially connected with mycelial strands of the ectomycorrhizal fungus *Thelephora terrestris*. Direct autoradiographic evidence of ¹⁴C transfer between *Pinus sylvestris* seedlings naturally connected by the mycelium of *Suillus bovinus* has been presented by BROWNLEE, *et al.* (1983). In this paper we present quantitative evidence of transfer in a range of ectomycorrhizal associations together with data relating to hyphal growth rates and the influence of source-sink relationships on inter-plant transfer.

MATERIALS AND METHODS

Ectomycorrhizas were synthesized aseptically in 250 ml Erlenmeyer flasks containing a peat-vermiculite (1:4 v/v) mixture moistened with modified Melin-Norkrans nutrient solution and inoculated with pure cultures of mycorrhizal fungi, isolated from fruit bodies. Infected and uninfected *Pinus* seedlings were transferred to perspex observation chambers containing non-sterile peat. In chambers containing infected plants extensive colonization of the peat by mycorrhizal mycelium occurred within 6-12 weeks

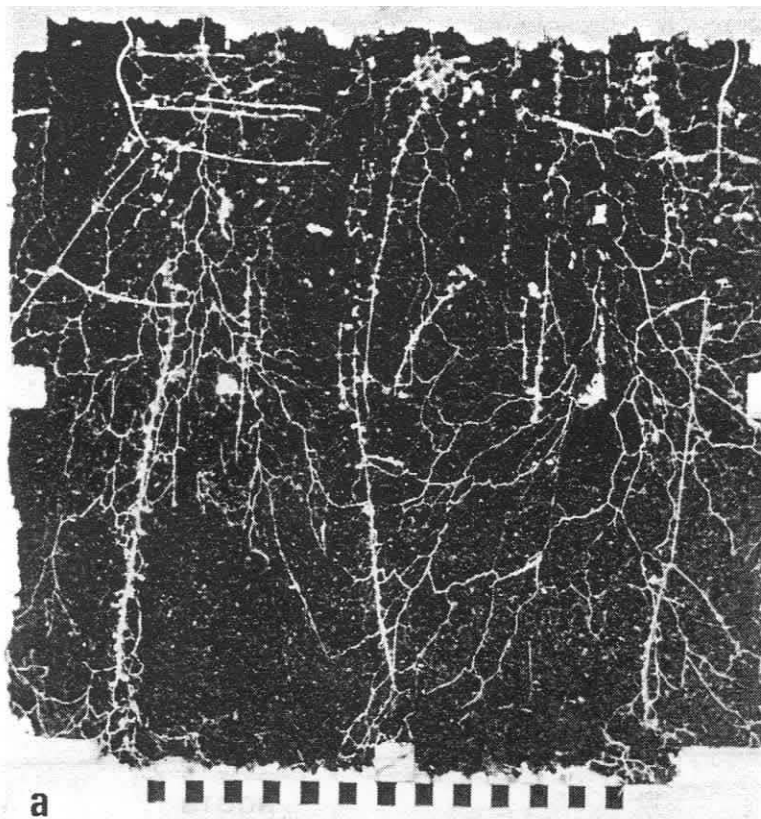
(1) Permanent address: Lund University, Department of Microbial Ecology,
Ecology Building, Helgonav.5, S-223 62 Lund, Sweden

after which time uninfected seedlings of the same or a related species were added to all chambers. In mycorrhizal chambers the roots of uninfected seedlings rapidly became infected giving rise to a network of mycelial connections between plants. Carbon transfer between plants was examined by exposing the shoots of older 'donor' plants to $^{14}\text{CO}_2$ in sealed chambers and monitoring the distribution of labelled assimilate through the mycelial network using autoradiography. Quantitative determination of isotope distribution to 'receiver' plants was achieved by grinding root and shoot material in liquid nitrogen, digesting aliquots of the extract in NCS tissue solubilizer and adding liquid scintillant prior to counting on a Packard liquid scintillation counter. The influence of shading on carbon transfer was examined by loosely shading certain plants in each chamber with aluminium foil for various periods prior to and during $^{14}\text{CO}_2$ feeding.

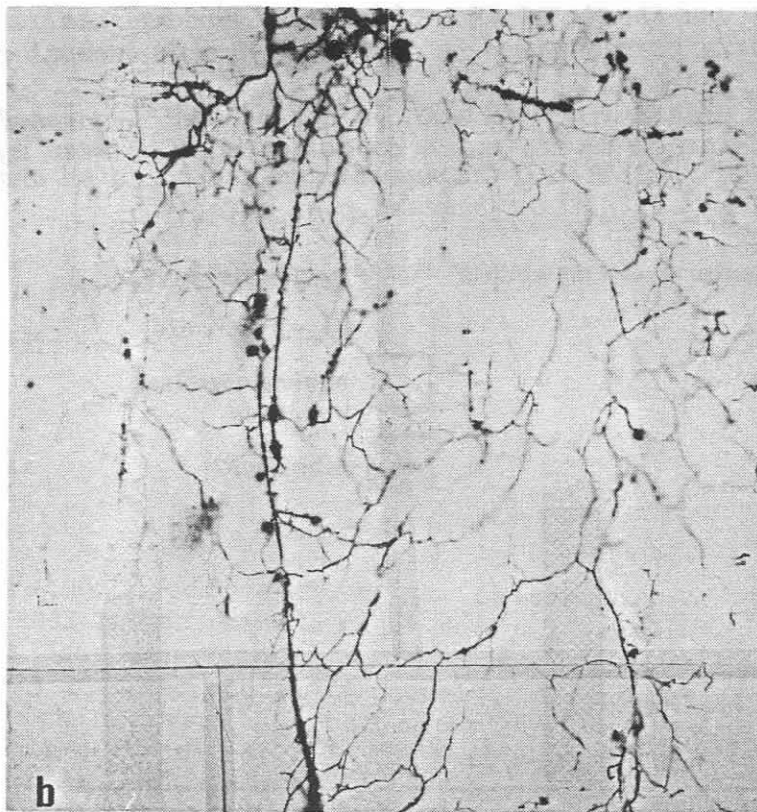
RESULTS

Colonization of chambers by mycelia of *Suillus bovinus* and *Pisolithus tictorius* spreading from infected roots was rapid and occurred at rates between 9 and 15 cm² per day. Mycorrhizal infection was rapidly initiated in uninfected roots as they made contact with spreading mycelium and subsequent aggregation of hyphae led to the formation of extensive networks of mycelial strands connecting plants (Plate a). Autoradiographic analysis of such systems (Plate b) shows that the interconnections provide direct pathways for the transfer of carbon between interlinked plants over distances exceeding 20 cm. Newly formed mycorrhizal roots of 'receiver' plants act as major sinks for the carbon and current assimilates of fed 'donor' plants are thus widely distributed to other plants with access to the same mycelium. Label accumulates in the sheath of mycorrhizal roots and is later transferred to the rest of the root system and shoots. Quantitative determination of the distribution of radioactivity within intra-specific associations of *Pinus contorta* infected by *Suillus bovinus* and *S. granulatus* (Fig. 1) shows that significantly larger amounts of label occur in the roots of 'receiver' plants grown in mycorrhizal chambers than in those of non-mycorrhizal plants where mycelial connections are absent. Levels of activity in the peat surrounding 'donor' roots did not differ significantly from background.

In chambers where inter-specific combinations of *P. contorta* and *P. sylvestris* were connected by a common mycelium the distribution of labelled compounds followed a similar pattern (Fig. 2). Significantly larger amounts of label accumulated in the roots of plants grown in mycorrhizal chambers than in those of plants grown in non-mycorrhizal chambers where levels of activity in 'receiver' plants were not significantly different from background levels. The effect of shading on carbon transfer was investigated in a range of ectomycorrhizal associations using chambers containing shaded and unshaded 'receiver' plants (Fig. 3). In chambers containing plants of *Pinus contorta* interconnected by *Suillus granulatus* significantly higher ($P < 0.01$) concentrations of radioactivity were found in the roots of shaded seedlings than in those which were of unshaded. In other mycorrhizal associations the differences between shaded and unshaded plants were not statistically significant but consistently higher mean levels of activity were found in shaded plants than in those which remained unshaded. These results suggest that the movement of label is strongly influenced by source-sink relationships and that levels of shoot irradiance may play an important role in determining the distribution of assimilate between the interconnected plants.



a



b

Plate 1-a) Root observation chamber containing *Pinus sylvestris*, *Pinus contorta* and *Pinus radiata* plants interconnected by mycelial strands of *Suillus bovinus*. The shoot systems of the plants have been removed.

b) Autoradiograph of the chamber shown in Plate a. A central plant has been fed with $^{14}\text{CO}_2$ and the labelled assimilate has been transferred from the roots of the fed plant to the adjacent plants *via* the mycelial network. The mycorrhizal roots of these plants act as strong sinks for activity.

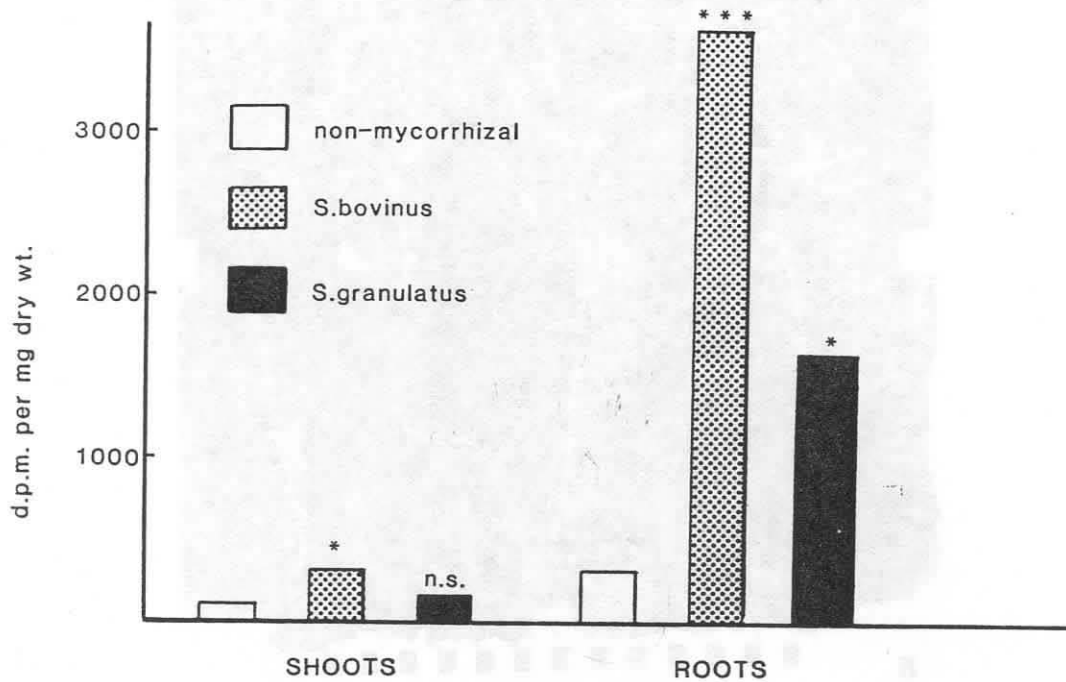


Figure 1. The distribution of radioactivity (d.p.m. per mg dry weight) in roots and shoots of mycorrhizal and non-mycorrhizal *Pinus contorta* plants grown in association with 'donor' plants of the same species.

Donor plants were fed with 50 μCi of $\text{NaH}^{14}\text{CO}_3$ for 72 hours. Significance levels refer to differences between mycorrhizal and non-mycorrhizal treatments and are based on analysis of variance of \ln transformed d.p.m. data.

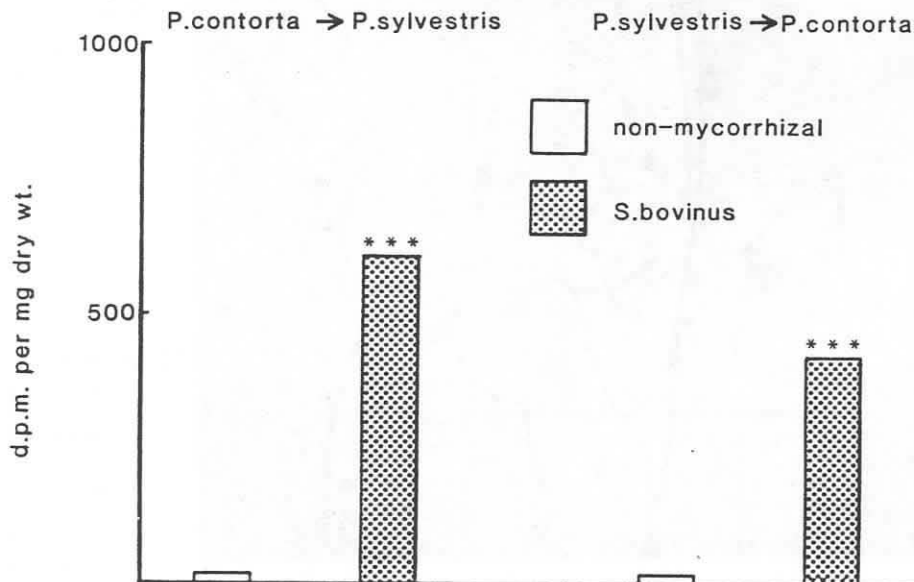


Figure 2. The distribution of radioactivity (d.p.m. per mg dry weight) in mycorrhizal and non-mycorrhizal roots of *Pinus contorta* and *Pinus sylvestris* grown in association with 'donor' plants of the opposite species. In each combination the 'donor' plant is the first named species.

Donor plants were fed with 10 μCi of $\text{NaH}^{14}\text{CO}_3$ for 96 hours. Significance levels refer to differences between mycorrhizal and non-mycorrhizal treatments and are based on analysis of variance of \ln transformed d.p.m. data.

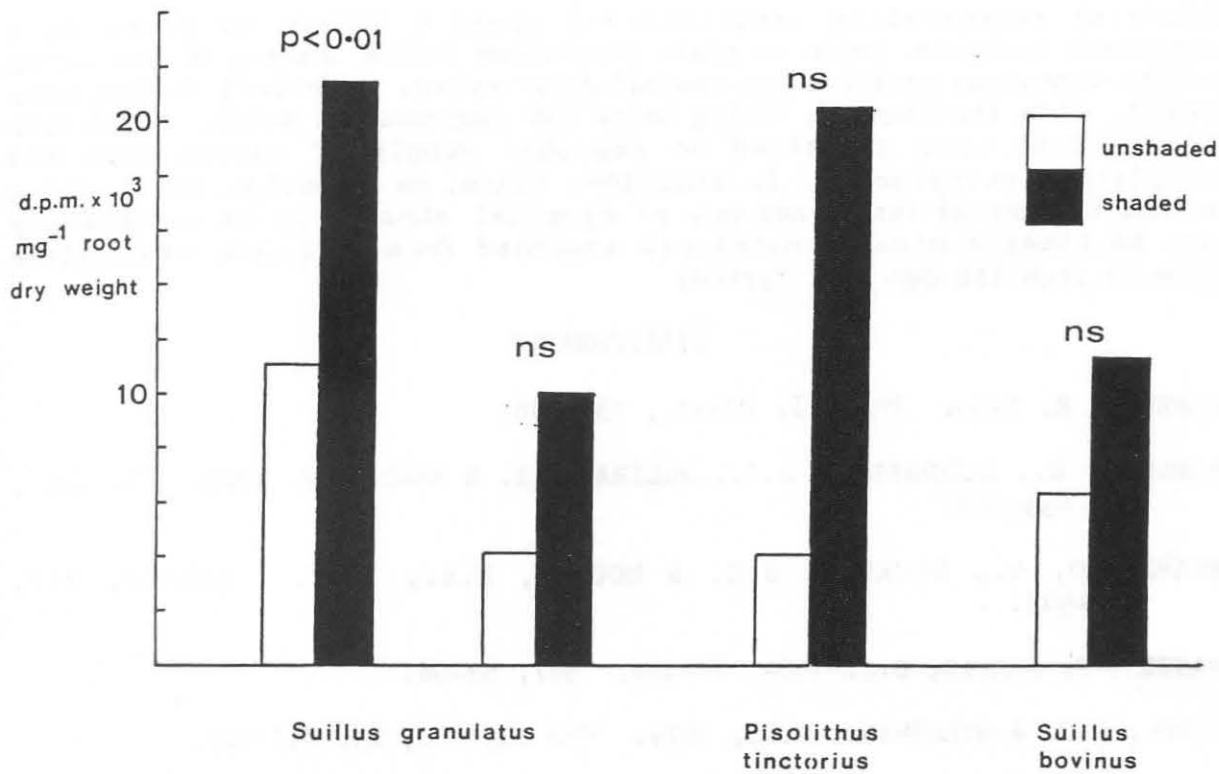


Figure 3. The distribution of radioactivity (d.p.m. per mg dry weight) in shaded and unshaded Pinus plants grown in association with donor plants infected with different ectomycorrhizal fungi.

Significance levels refer to differences between shaded and unshaded treatments based on blocked analysis of variance of \ln transformed d.p.m. data.

DISCUSSION

The present study confirms the observation of BROWNLEE *et al.* (1983) that, in addition to being a major source of inoculum, the mycelial system provides conduits through which carbon flows from an established food base to newly infected roots. Clearly, without net carbon flow, the heterotroph, which has very limited saprotrophic capability, would be unable to spread through the soil. The presence of functional mycelial connections, provides the potential for flow along gradients so that areas of low substrate availability can be supplied with assimilate. Factors such as irradiance are likely to determine assimilate supply to roots in nature and the experimental data suggest that manipulation of sink size by shading 'receiver' plants can lead to enhanced carbon transfer. The situation is thus similar to that observed by FRANCIS & READ (1984) in VA mycorrhizas.

The ecological significance of the formation of mycelial plant interconnections has been discussed by READ *et al.* (1985) who point out that the newly revealed carbon pathway might be critically important for the survival of seedlings growing in the shade of adult overstorey plants.

Naturally regenerating seedlings can spend a number of years in a suppressed condition below an adult population before opening of the canopy permits sustained positive net assimilation rates. Survival during this period, when the plant is living below its compensation point, might well be dependent upon sustained or seasonal supply of carbon from the illuminated overstorey. In addition, since, on infection, the seedling becomes integrated into a network of mycelial strands it is potentially able to receive mineral nutrients absorbed from soil at a considerable distance from its own root system.

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